The HLA Class I Restricted CTL Response in HIV-1 Infection: Systematic Identification of Optimal Epitopes

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I. Introduction

Viral infection induces CD8+ cytotoxic T lymphocytes (CTL) responses by presenting viral antigen on MHC class I molecules at the surface of infected cells (1). A vigorous CTL response is also associated with Human Immunodeficiency Virus (HIV) infection (2). HIV specific CTL are thought to play an important role in the control of the virus during clinical latency and may influence the course of disease development (3-6). Furthermore, experiments in HIV-exposed, uninfected individuals suggest that CTL could provide protection from infection (7-9). The characterization of the antigenic epitopes involved in CTL induction is important not only for a better understanding of disease pathogenesis, but also for possible vaccine development. Since the first description of HIV specific CTL, the efforts of many laboratories have led to the identification of a large number of epitopes involved in the HIV-1 specific CTL response in infected persons(10, 11). A summary of those sequences is accessible in this database. It contains more than 200 peptides, which contain putative HLA class I restricted epitopes. In many cases, these peptides require further definition as to the minimal epitope or the restricting HLA molecule. Here, we have compiled a list of peptides that have been defined in terms of the minimal length that mediates the most effective target cell sensitization in the context of the restricting class I molecule. More than 60 peptides fulfilling these criteria were included in the last database update (11). Within the last 12 months, more than 30 new optimal epitopes have been identified, many of which are not yet published or are in submitted reports.

II. Presentation and recognition of viral antigen on MHC class I

Recent advances in the understanding of MHC class I antigen processing have provided insights into peptide selection for CTL responses. Viral antigen is presented to specific T cells as short peptides in association with MHC molecules (1, 12). These antigenic epitopes are derived from viral cytosolic proteins which undergo proteasome mediated processing (3, 13). A number of parameters likely influence peptide processing, including amino acids both within and outside the CTL epitope in the cytosol(14). Following processing, the peptides are translocated into the lumen of the ER by the TAP1/TAP2 heterodimer and bound to nascent MHC class I molecules. Both interactions (TAP and MHC) seem to have restrictive specificities and to allow only some peptides to be presented (3, 13, 15). Whereas peptide binding to MHC molecules has been extensively studied, both the impact of different TAP-alleles on the generation of peptides and the link between TAP alleles and HLA alleles are unclear (15-17). As revealed by peptide titration studies and peptide elution from the isolated MHC class I molecule, HLA class I restricted epitopes normally are about 8-12 amino acids in length and have certain anchor residues to bind into specific pockets of the MHC class I molecule. These pockets, designated A, B, C, D, E and F, are located in the antigen binding site on the class I molecule formed by two alpha helices, with the floor of the groove formed by a beta-sheet structure (18). As the pockets are located at the edge of the peptide binding groove, the anchor positions in the peptides are often in positions 1, 2 and 9 of the processed peptide (19). However, other residues can influence the peptide binding as well (20). Eluting peptides from various MHC class I molecules has led to the definition of MHC-allele-specific binding motifs (19-21). These motifs can be used to screen protein sequences in order to find potentially antigenic epitopes presented by a certain HLA allele (22). However, this approach only reflects peptide binding but not necessarily their involvement in the natural CTL response *in vivo*, as such evaluations do not analyze the effects of protein processing and transportation. Very

recently, the x-ray crystal structure and orientation of a T cell receptor (TCR) bound to a MHC/peptide complex was described (23). The V region of the α and the β chains, resembling antibody structures, contacts the presented peptide in its core region through the third complementarity-determining regions (CDR 3), forming a deep hydrophobic cavity. The CDR1 and CDR2 regions of the α-chain appear to contact the MHC/peptide complex around the N-terminal end of the embedded peptide, whereas the β chain CDR1 and CDR2 contact the C-terminus of the peptide. These findings may help to explain the biased use of certain V-β chain segments observed in various immune responses, i.e. influenza infection and different autoimmune diseases (24, 25).

III. Identification of HIV-1 derived CTL epitopes

Various methods have been used to identify HIV-1-derived, HLA class I restricted epitopes. The majority of epitopes have been defined using CTL lines or clones recognizing recombinant viral antigen, such as target cells infected with vaccinia virus constructs expressing selected HIV-1 proteins. The minimal epitope for these responses is determined by using synthetic peptides, followed by titration assays with truncated peptides to identify the optimal epitope (26). Alternatively, binding-motif based searches for potential epitopes has led to the identification of some optimal epitopes (22). However, to prove that the binding peptide is involved in the natural immune response to HIV, recognition of the naturally processed peptide by CTL in infected persons is required. Finally, elution of peptide from HLA class I molecules allows for the direct isolation of processed, class I binding epitopes (27). However, these epitopes are not necessarily immunogenic *in vivo* and may thus not induce a CTL response in infected humans.

In published studies, different methods have been used to identify optimal epitopes. In some cases the HLA restriction has not been shown or the optimal epitope has not been defined by titration assays using truncated peptides. The Los Alamos HIV Molecular Immunology Database contains all reported sequences with CTL activity, regardless of their definition as optimal epitopes. However, for many different questions and approaches such as the design of a epitope based vaccine, the knowledge of the optimal epitope is a prerequisite. In the list presented here (Table 1), we include only those peptides which fit rigid criteria: 1) the CTL recognize the naturally processed epitope, 2) titration assays with truncated peptides have identified the optimal epitope and 3) the restricting HLA molecule has been defined. A number of likely optimal epitopes were not included as these criteria were not met. For example, we did not include putative HLA-Cw8 restricted epitopes, since Cw8 is in a strong linkage disequilibrium with HLA-B14 and it is thus difficult to identify the restricting HLA allele by using (mis)matched cell lines (28, 29). However, these sequences can be found in other parts of the database.

Table 1 Best Defined HIV CTL Epitopes

HLA	Protein	AA	Isolate	Sequence	Reference	Ref No*
HLA-A2				2 C	Falk 91	21
				L V		
				I L		
	p17	77–85	LAI	SLYNTVATL	Johnson 91, Parker 92,94	35,36,37
	RT	346–354	LAI	VIYQYMDDL	T. Harrer	74
	RT	476–484	LAI	ILKEPVHGV	Walker 89, Tsomides 91	38,39
	gp41	818–827	LAI	SLLNATDIAV	Dupuis 95	40
	nef	136–145	LAI	PLTFGWCYKL	Haas 96, B. Maier and B. Autran	41,PC
	nef	180–189	LAI	VLEWRFDSRL	Haas 96, B. Maier and B. Autran	41,PC
	nef	190–198	LAI	AFHHVAREL	Hadida 95, B. Autran	42,PC

Table 1 (cont.) Best Defined HIV CTL Epitopes

HLA	Protein	AA	Isolate	Sequence	Reference	Ref No*
HLA-A3.1				23 C	Di Brino 93	43
				IF K		
				L Y		
	p17	18–26	LAI	KIRLRPGGK	Harrer 96	44
	p17	20–28	LAI	RLRPGGKKK	B. Culmann, D. Lewinsohn, S. Riddell	PC
	p17	20–29	LAI	RLRPGGKKKY	B. Wilkes, D. Ruhl	PC
	RT	325–333	LAI	AIFQSSMTK	S. Threlkeld	S
	gp120	37–46	LAI	TVYYGVPVWK	Johnson 94 b	45
	gp41	775–785	LAI	RLRDLLLIVTR	Takahashi 91	46
	nef	73–82	LAI	QVPLRPMTYK	Koenig 90, Culmann 91	47,48
HLA-A11				2 C	Zhang 93	49
				I K		
				L		
	p17	84–92	LAI	TLYCVHQRI	T. Harrer	PC
	p24	349–359	III-B	ACQGVGGPGGHK	Sipsas 96	29
	RT	325–333	LAI	AIFQSSMTK	Johnson 94 a, Zhang 93, S. Threlkeld	50,49,S
	RT	508–517	LAI	IYQEPFKNLK	B. Culmann	PC
	nef	73–82	LAI	PLRPMTYK	Culmann 91	48
	nef	84–92	LAI	AVDLSHFLK	Culmann 91	48
HLA-A19						
A*7401	RT	71–79	Clade A/B/D	ITLWQRPLV	S. Rowland-Jones	PC
HLA-A24						
	p17	28–36	LAI	KYKLKHIVW	D. Lewinsohn	PC
	gp120	53–62	LAI	LFCASDAKAY	Lieberman 92, Shankar 95	51,52
	gp41	591–598	LAI	YLKDQQLL	Dai 92	53
HLA-A25						
	p24	145–155	LAI	QAISPRTLNAW	I. Kurane, K. West	PC
	p24	203–212	LAI	ETINEEAAEW	Klenerman 96, C. Van Baalen 96	54,55
HLA-A26						
	p24	167–175	LAI	EVIPMFSAL	P. Goulder	IP–AIDS
	RT	593–603	LAI	ETFYVDGAANR	B. Wilkes, D. Ruhl	PC
HLA-A28						
A*6802	RT	71–79	Clade A/B/D	ITLWQRPLV	S. Rowland-Jones	PC
A*6802	RT	85–93	Clade D	DTVLEEMNL	S. Rowland-Jones	PC

Table 1 (cont.) Best Defined HIV CTL Epitopes

HLA	Protein	AA	Isolate	Sequence	Reference	Ref No*
HLA-A29						
	gp120	376–384	LAI	FNCGGEFFY	C. Wilson	IP–JV
HLA-A31						
	gp41	775–785	LAI	RLRDLLLIVTR	Safrit 94 a, Safrit 94 b	56,57
HLA-A32						
	RT	559–568	LAI	PIQKETWETW	Harrer 96	44
	gp120	419–427	HXB2	RIKQIINMW	Harrer 96	44
HLA-B7				123 C APR L	Engelhard 93	58
	p24	148–156	LAI	SPRTLNAWV	D. Lewinsohn	PC
	p24	179–187	LAI	ATPQDLNTM	B. Wilkes, D. Ruhl	PC
	gp120	303–312	LAI	RPNNNTRKSI	J. Safrit and R.A. Koup	PC
	gp41	843–851	LAI	IPRRIRQGL	B. Wilkes	PC
	nef	68–77	LAI	FPVTPQVPLR	Haas 96, B. Maier and B. Autran	41,PC
	nef	128–137	LAI	TPGPGVRYPL	Culmann 94, Haas 96, B. Maier and B. Autran	59,41,PC
HLA-B8				3 5 C K K I	Hill 92, Sutton 93	60,61
	p17	24–31	LAI	GGKKKYKL	Rowland-Jones 93, S. Reid	62,U
	p17	74–82	LAI	ELRSLYNTV	P. Goulder	S
	gp120	2–10	III-B	RVKEKYQHL	Sipsas 96	29
	gp41	591–598	LAI	YLKDQQLL	Johnson 92, Shankar 95	63,52
	nef	13–20	LAI	WPTVRERM	P. Goulder	S
	nef	90–97	LAI	FLKEKGGL	Culmann 94, P. Goulder	59,PC
HLA-B14				23 56 C RL RI L KY HL F	Di Brino 94	64
	p24	183–191	LAI	DLNTMLNTV	Nixon 88, Johnson 92	65,63
	p24	298–306	LAI	DRFYKTLRA	Harrer 96	44
	gp41	589–597	LAI	ERYLKDQQL	Johnson 92	63
HLA-B15						
	gp120	375–383	LAI	SFNCGGEFF	C. Wilson	73
HLA-B18						
	nef	135–143	LAI	YPLTFGWCY	Culmann 91, Culmann 94	48,59

Table 1 (cont.) Best Defined HIV CTL Epitopes

HLA	Protein	AA	Isolate	Sequence	Reference	Ref No*
HLA-B27				2 C R K R	Jardetzky 91	66
	p17	18–27	LAI	KIRLRPGGKK	D. Lewinsohn	PC
	p17	19–27	LAI	IRLRPGGKK	D. Lewinsohn	PC
	p24	263–272	LAI	KRWIILGLNK	Nixon 88 b, Buseyne 93	67,68
	gp41	590–597	LAI	RYLKDQQL	Shankar 95	52
	gp41	791–799	LAI	GRRGWEALKY	Liebermann 92, J. Liebermann	51,PC
	nef	73–82	LAI	QVPLRPMTYK	B. Culmann	PC
HLA-2705	nef	105–114	LAI	RRQDILDLWI	P. Goulder	S
	nef	134–141	LAI	RYPLTFGW	B. Culmann	PC
B*2703	gag	260–269	HIV-2	RRWIQLGLQK	S. Rowland-Jones	PC
HLA-B35				2 C P Y S	Hill 92	60
	p17	36–44	LAI	WASRELERF	P. Goulder	S
	p17	124–132	LAI	NSSKVSQNY	Rowland-Jones 95	69
	p24	254–262	LAI	PPIPVGDIY	Rowland-Jones 95	69
	RT	262–270	LAI	TVLDVGDAY	B. Wilkes, D. Ruhl	PC
	RT	273–282	III-B	VPLDEDFRKY	Sipsas 96, Shiga 96	29,34
	RT	328–336	III-B	NPDIVIYQY	Sipsas 96, Shiga 96	29,34
	RT	342–350	LAI	HPDIVIYQY	Rowland-Jones 95	69
	gp120	42–52	LAI	VPVWKEATTTL	B. Wilkes, D. Ruhl	PC
	gp41	611–619	LAI	TAVPWNASW	Johnson 94 b	45
	nef	74–81	LAI	VPLRPMTY	Culmann 91, Culmann 94	48,59
	gag	245–253	HIV-2	NPVPVGNIY	Rowland-Jones 95	69
HLA-B37						
	nef	120–128	LAI	YFPDWQNYT	Culmann 91	48, PC
HLA-B39						
	p24	193–201	LAI	GHQAAMQML	I. Kurane, K. West	PC
HLA-B42						
	p17	20–29	LAI	RLRPGGKKKY	B. Wilkes, D. Ruhl	PC
	RT	438–446	LAI	YPGIKVRQL	B. Wilkes, D. Ruhl	PC
HLA-B45						
	RT	591–600	LAI	GAETFYVDGA	B. Wilkes, D. Ruhl	PC

Table 1 (cont.) Best Defined HIV CTL Epitopes

HLA	Protein	AA	Isolate	Sequence	Reference	Ref No*
HLA-B51						
	p24	325–333	LAI	NANPDCKTI	B. Wilkes, D. Ruhl	PC
	RT	295–302	III-B	TAFTIPSI	Sipsas 96	29
	gp41	557–565	III-B	RAIEAQQHL	Sipsas 96	29
HLA-B52						
	p24	275–282	LAI	RMYSPTSI	B. Wilkes, D. Ruhl	PC
HLA-B53				2 C		60
				P Y	Hill 92	
				F		
				W		
	HIV-2 gag	173–181	HIV-2	TPYDINQML	Gotch 93	70
HLA-B55						
	gp120	42–51	LAI	VPVWKEATTT	Shankar 95	52
HLA-B57						
	p24	147–155	III-B	ISPRTLNAW	Johnson 91, P. Goulder	35,IP–ARHR
	p24	140–149	LAI	TSTLQEQIGW	P. Goulder	IP–ARHR
	p24	162–172	LAI	KAFSPEVIPMF	P. Goulder	IP–ARHR
	p24	240–249	LAI	TSTLQEQIGW	P. Goulder	PC
	p24	311–319	LAI	QASQEVKNW	P. Goulder	IP–ARHR
	p24	311–319	LAI	QASQDVKNW	P. Goulder	IP–ARHR
	nef	116–125	LAI	HTQGYFPDWQ	Culmann 91	48, PC
	nef	120–128	LAI	YFPDWQNYT	Culmann 91	48, PC
HLA-B58						
	p24	140–149	LAI	TSTLQEQIGW	P. Goulder	IP–ARHR
HLA-Bw62						
	p17	20–29	LAI	RLRPGGKKKY	Johnson 91, B. Wilkes, D. Ruhl	35,PC
	p24	268–277	LAI	LGLNKIVRMY	Johnson 91	35
	RT	415–426	III-B	LVGKLNWASQIY	P. Johnson	PC
	RT	476–485	LAI	ILKEPVHGVY	Johnson 91, P. Johnson	35,PC
	nef	84–91	LAI	AVDLSHFL	Culmann 94	59
	nef	117–127	LAI	TQGYFPDWQNY	B. Culmann	PC
HLA-Cw01.02						
	p24	168–175	LAI	VIPMFSAL	P. Goulder	S

Table 1 (cont.) Best Defined HIV CTL Epitopes

HLA	Protein	AA	Isolate	Sequence	Reference	Ref No*
HLA-Cw4				2 C	Falk 94	71
				Y F		
				P L		
				F M		
	gp120	380–388	LAI	SFNCGGEFF	Johnson 93, C. Wilson	72,73

*In Ref No column the following abbreviations apply:
PC = personal communication
S = submitted
IP-AIDS = in press - *AIDS*
IP-JV = in press - *Journal of Virology*
IP-ARHR = in press - *AIDS Research and Human Retroviruses*

IV. Analysis of HIV-1 derived CTL epitopes

The list of HIV derived CTL epitopes will continue to grow, as new epitopes are found and longer sequences are mapped to the optimal epitope. The list given in Table 1 should provide investigators with a reliable compilation of optimal CTL epitopes in HIV. With the growing number of defined optimal epitopes, some interesting characteristics are beginning to emerge. More epitopes which overlap with previously described ones have been identified, as have epitopes which can be restricted by multiple HLA alleles. These latter epitopes could be interesting candidates for peptide based vaccine development. This is the case for RT protein derived epitope AIFQSSMTK (a.a.325- a.a.333) which binds to HLA-A3 and HLA-A11, both members of the same binding motif superfamily (30). The gag-p17 derived peptide RLRPGGKKKY (a.a. 20-29) was found to be presented by at least three different HLA alleles (HLA-A3, -B42, -Bw62). Additional examples of epitopes binding to multiple HLA molecules are included in this list and can be conveniently found by searching the Los Alamos Database. Furthermore, it becomes clear that some areas contain multiple overlapping peptides. By minute alteration (i.e. truncation by or substitution of one amino acid), those epitopes may be able to bind to additional HLA molecules without losing the binding affinity to the original HLA molecule(s) nor escaping recognition by CTL specific for the original peptide. Different studies have shown that there can be wide cross recognition of variants of certain epitopes (8, 31, 32). This would suggest that a less conserved, but highly immunodominant epitope may serve as a superior immunogen for vaccine development than one which is highly conserved, but only rarely recognized. However, one must consider the potential adverse effects of sequence variation within, or flanking, these epitopes which may alter peptide processing and presentation. In general, it seems that the induction of a strong CTL response requires epitopes that bind tightly to the class I molecule. It has been suggested that the immunogenicity of potential CTL epitopes can be predicted more accurately by the dissociation rate than by the binding affinity (33). Although Table 1 lists numerous epitopes, little is known about their potential immunodominance, which may be especially important with regard to the development of peptide based vaccines (32, 34). The mechanisms leading to such immunodominance are not clear, although several reasons have been discussed. These include the preferential processing of certain epitopes by the proteasome due to favored flanking regions, the binding affinity to the class I molecule, the peptide selectivity of the TAP dimer, and a lack in the T cell repertoire. Thus, epitopes selected as peptide vaccine candidates may need to be tested not only for variability and binding affinities but rather for parameters like dissociation rate and immunodominance.

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